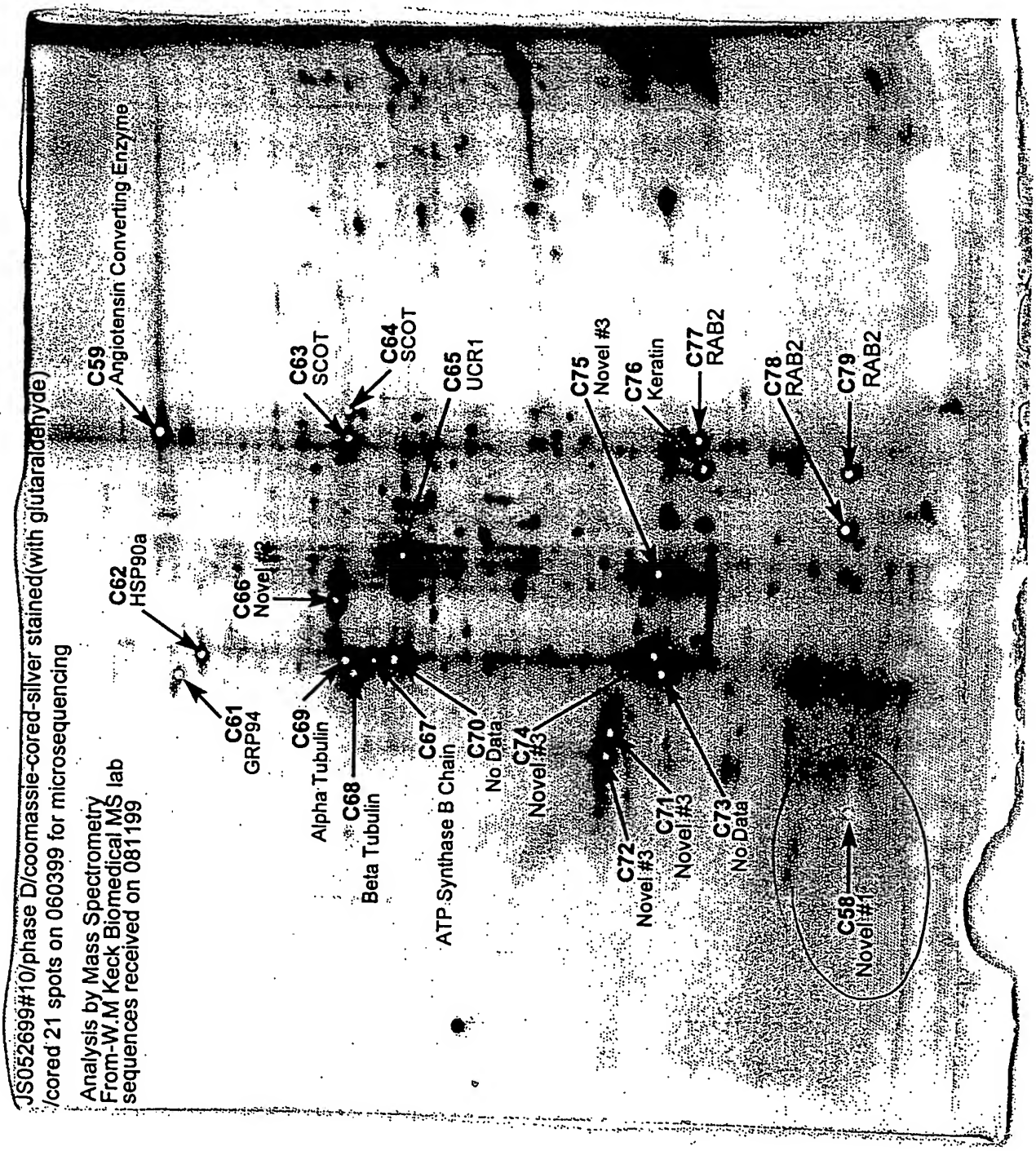
Name: Jagannath Sneh Date: 8/12/04

Experiment:

039



10/809,654 BEST AVAILABLE COPY EXHIBIT 1

Name: Jagathpala Sneth Date: 8/15/9,

Experiment:

Report number: 400
Sequence Analysis of 22 2D Gel Bands.
8/11/99

Band C58. The peptides shown in Table 1 were detected in Band C58 (LB6-43-1). These peptides belong to Novel #1.

Table 1. Peptide sequences from Band C58 (LB6-43-1).

Peptide No.	Measured M W ($M+H^+$, Da)		Peptide sequence by CAD[1]
1	≈1482.8	+2	ATSC[a]GLEEPVSYR
2	1499.4	+2	ATSC[a](o)GLEEPVSYR
3	5033.8	+5	- - - XSDSMEC[a] - - -
4	5049.7	+5	- - - XSDSM(o)EC[a] - - -

GLEEPVSYR ~ 9mer

[1]I and L cannot be distinguished by low energy CAD but are inferred by the database sequence, M(o) designates oxidized M, C is carbamidomethyl modified unless noted as C[a] (acrylamide), _ designates a single unknown residue, - - - designates an unknown number of unknown residues.

Name: Jagathy La Shetty Date: 8/1.5/99

Experiment:

042

Nucleotide and deduced amino acid sequence of Human Testis EST (Ac. # AA778671) which matched to Tryptic peptide obtained by Mass spectrometry of c

Soares Testis NHT Homo sapiens cDNA clone 1049023
mRNA sequence.
ACCESSION AA778671

```
     GCACTGGTCCGGTCATCAACAAAGGCTGCCTGCGAGCCACCAGCTGCGGCCTTGAGGAAC
1    ---------+---------+---------+---------+---------+---------+ 60
       T  G  P  V  I  N  K  G  C  L  R  A  T  S  C  G  L  E  E  P

     CCGTCAGCTACAGGGGCGTCACCTACAGCCTCACCACCAACTGCTGCACCGGCCGCCTGT
61   ---------+---------+---------+---------+---------+---------+ 120
       V  S  Y  R  G  V  T  Y  S  L  T  T  N  C  C  T  G  R  L  C

     GTAACAGAGCCCCGAGCAGCCAGACAGTGGGGGCCACCACCAGCCTGGCACTGGGGCTGG
121  ---------+---------+---------+---------+---------+---------+ 180
       N  R  A  P  S  S  Q  T  V  G  A  T  T  S  L  A  L  G  L  G

     GTATGCTGCTTCCTCCACGTTTGCTGTGACGAACAGGGAGGACAGGGCCTGGGACTGTTC
181  ---------+---------+---------+---------+---------+---------+ 240
       M  L  L  P  P  R  L  L  *  P  T  G  R  T  G  P  G  T  V  L

     TCCCAGATCCGCCACTCCCCATGTCCCCATGTCCTTCCCCCACTAAATGGCCAGAGAGGC
241  ---------+---------+---------+---------+---------+---------+ 300
       P  D  P  P  L  P  M  S  P  C  P  S  P  T  K  W  P  E  R  P

     CCTGGACAACCTCTTGCGGCCCTGGCTTCATCCCTTCTAAGGCTGTCCACCAGGAGCCCG
301  ---------+---------+---------+---------+---------+---------+ 360
       W  T  T  S  C  G  P  G  F  I  P  S  K  A  V  H  Q  E  P  G

     GTGCTAGGGGAAGCATCCCCAGGCCTGACTGAGCGGCAGGGGAGCACGGCCCGTGGGTTT
361  ---------+---------+---------+---------+---------+---------+ 420
       A  R  G  S  I  P  R  P  D  *  A  A  G  E  H  G  P  W  V  *

     GATTGTATTACTCTGTTCCACTGGTTCTAAGACGCAGAGCTTCTCACATCTCAATCAGGA
421  ---------+---------+---------+---------+---------+---------+ 480
       L  Y  Y  S  V  P  L  V  L  R  R  R  A  S  H  I  S  I  R  M

     TGCTTCTCTCCATTGGTAGCACTTTAGAGTCCATGAAATATGGTAAAAAATATATATATA
481  ---------+---------+---------+---------+---------+---------+ 540
       L  L  S  I  G  S  T  L  E  S  M  K  Y  G  K  K  Y  I  Y  I

     TCATAATAAATGACAGCTGATGTTCAAAA
541  ---------+---------+--------- 569
       I  I  N  D  S  *  C  S  K
```


Important: Place card under blue conv.

EXHIBIT 3

Name: Jagathpali Shetty Date: 05/26/9,

Experiment: PCR to generate C58 - partial cDNA

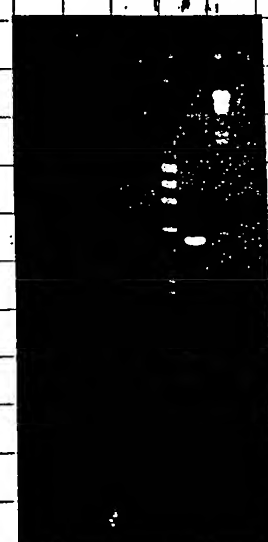
PCR with primers for C58 - EST

using both forward and reverse primer.

Bottom:		Top:
3.025	3.3 Bf	4.15
2	4 dNTP	
2	Mg	
1.25	C5PF (C58-F-EST)	
1.25	C5PR (C58-R-EST)	
0.475	H_2O	7.95
	cDNA	2
	polyase	0.5

PCR programme

1. 94 2:30
2. 94 :30
3. 68 :30 Δ -1.5/cycle
4. 68 2:30
5. Goto 2 (11X)
6. 94° :30
7. 50° :30
8. 68 2:00
9. Goto 6 (27X)
10. 68 18:00
11. 4° ∞



Result: Obtained a product around 520 bp. which matched to the expected product i.e. 519 bp

Name: Jagathpur Sheth Date: 9/7/99

Experiment:

The Sequence for C58 EST was obtained from the Sequencing Lab.

Sequence of PCR-derived EST 9/7/99

partial sequence for C58

```
!!NA_SEQUENCE 1.0
Sequence of PCR-derived EST from 9/7/99
c58est.dna  Length: 475  September 7, 1999 12:00  Type: N  Check: 5379  ..

      1  CTGCGGCCTT GAGGAACCCG TCAGCTACAG GGGCGTCACC TACAGCCTCA

     51  CCACCAACTG CTGCACCGGC CGCCTGTGTA ACAGAGCCCC GAGCAGCCAG

    101  ACAGTGGGGG CCACCACCAG CCTGGCACTG GGGCTGGGTA TGCTGCTTCC

    151  TCCACGTTTG CTGTGACCAA CAGGGAGGAC AGGGCCTGGG ACTGTTCTCC

    201  CAGATCCGCC ACTCCCCATG TCCCCATGTC CTTCCCCCAC TAAATGGCCA

    251  GAGAGGCCCT GGACAACCTC TTGCGGCCCT GGCTTCATCC CTTCTAAGGC

    301  TGTCCACCAG GAGCCCGGTG CTAGGGGAAG CATCCCCAGG CCTGACTGAG

    351  CGGCAGGGGA GCACGGCCCG TGGGTTTGAT TGTATTACTC TGTTCCACTG

    401  GTTCTAAGAC GCAGAGCTTC TCACATCTCA ATCAGGATGC TTCTCTCCAT

    451  TGGTAGCACT TTAGAGTCCA TGAAA
```

Name: Jagathpala Sheth Date: 9/7/19. Genetic CDNA

Experiment: Cloning of C58 (Screening of Library) 050

A culture of K 802 strain host ~~C58~~ was made.

medium used : NZCYM medium:

2 ml of NZCYM + 20 µl of 20% maltose soln (Actual conc is 0.2% in the medium)

K 802 Cells host strain taken from -70°C with a sterile tip taken out and placed inside the medium.

Kept at 37°C - Shaker.

Name: Jagathpala Sheth Date: 09/08/99.

Experiment: Cloning of C58

051

Titration of the λ DR2 Library.

(λ DR2 - Human Testis cDNA Library)

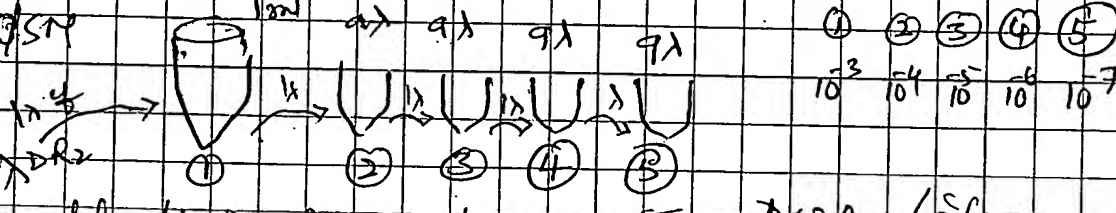
① One NZCYM medium was thawed using microwave.

② About 20 ml each of the medium was plated on 5 plates and the cap was kept open (in the Sterile Hood)

③ Mean time Take the culture of K802 left at 37°C previous day.

~~and Take~~

④ Take 1ml of SM buffer (buffer for λ DR2 i.e. phage buffer) in a tube. and 9µl each to 4 other tubes.



add 1λ of λDR2 human T cDNA Library to the first tube. Vortex.

Take 1λ from tube 1 to tube 2, vortex and take 1λ from #2 & transfer to 3 and so on. Vortex.

Take 1λ each from each tube and to a 10 ml tube (round bottom).

Important

Laboratory Research

National® Brand

Name: Jagathpala Shetty Date: 09/08/99

Experiment:

(5) Add 75λ each of the K802 culture to all tubes. - Wait for 20 minutes.

(6) Keep three Raw N2 Cyne - agarose (0.7%?) medium and allow it to come to ≈ 50°C (for the top layer)

(7) Keep a water bath at 37°C with a thermometer.

(8) Keep the tubes at 37°C for 2 minutes

(9) Take ≈ 4 ml of the melted ~~agar~~ N2 Cyne agarose - in to the tubes containing 1 ml of the Contents from the tubes to the LB Agar plates, Swirl the plates as you pour. Allow it to cool for 10 min. to allow the inoculum to soak in to agar.

(10) Incubate plates at 37°C O/N.

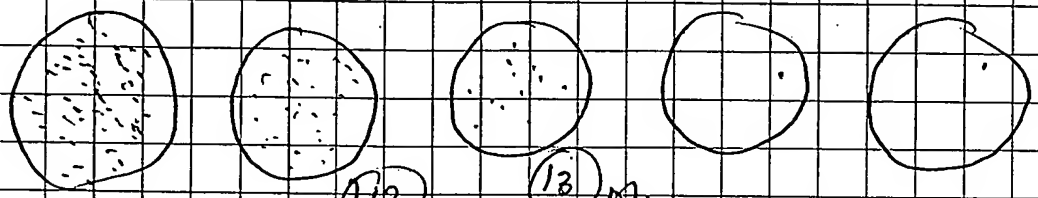
Name: Jegathgaler Sneh Date: 09/09/19

Experiment: Screening of Library

053

The plaques on the plates excerpted.

	①	②	③	④	⑤
dil	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}



213 pf

13 pf

#① i.e: too many

#② 213×10^4 i.e. $2.13 \times 10^6/\lambda$

#③ 13×10^5 i.e. $1.3 \times 10^6/\lambda$

average $\simeq 1.7 \times 10^6/\lambda$

average phage to be used for screening : $\simeq 40 \times 10^4$

1 λ dilute to 100 λ

i.e: $\lambda \rightarrow 17 \times 10^3$

can take $\simeq 2.5\lambda$ i.e gives $\simeq 50 \times 10^3$ phage

Name: Jagathpala Shetti
Experiment:
Date: 09/09/99

054

Transfection of Host Strains

Poured 6 bigger plates with NZCYM medium
poured 50 ml each (1.3% agar)

Taken a small crystal of library ~~xx~~
λDR2 from -70°C and the stock kept
back.

Take 1λ → dil 100λ

20.5λ → should give ≅ 50,000 phages

The bugs in 10 ml of NZCYM with 2% maltose
— Spun → pellet taken and
resuspended in ~~10~~ 5 ml of 10 mM
$MgSO_4$

Taken 600λ ~~each for in 6~~ tubes
of bugs + 15λ of phage.

620λ of cells
15λ of phage (1:100 diluted)

↓ 20 minutes

↓ Take 100λ each and
add to 6 tubes
Mean time top agarose at 50-55°C

Name: Jagathpala Sheth Date: 09/09/99

Experiment: screening of Library

056

DNA labelling

Protocol: Feinberg & Vogelstein Method

TO a sterile microfuge add:

C-58-EST	DNA	(≈50ng)	ie: 2µl
	H_2O		31.5
	Boil → 5min		
oligolabeling buffer	OLB		10λ
	[α^{32}P] dCTP		5λ
	Klenow		1.5λ

* After adding OLB keep at −20 for a while.

Add 5λ of α^{32}P dCTP and 1.5λ of Klenow. Tackle for a while and leave at 37°C.

Name: Jagathpala Sethi Date: 09/10/99.

Experiment: Cloning of CSB- Contd. (Library Screening) 057

The plates - taken out from 37°C and chilled at 4°C.

Membrane lifting.

① The nylon membranes - 6 of them membrane and 3 marks - were done at 3 corners - randomly.

② Membrane - placed on the plate carefully in one attempt. (Do not lift and change the position) - Leave for 2 min. (using tweezers)

③ Lift - make 5 marks with syringe needle. also make mark on the side of the plate corresponding to pencil mark on filter. Take the membrane carefully from one end and by using tweezer and place it on a whatman filter paper soaked with denaturation soln - 5-10 min. Film should be placed phage side up. Change positions in order ensure the complete immersion of the filter in the solution.

④ Place the membrane on a whatman paper containing Neutralization buffer. Change positions - ensure complete immersed and - 5-10 minutes

⑤ Cross-linking:

① Place inside on a whatman

② press power on

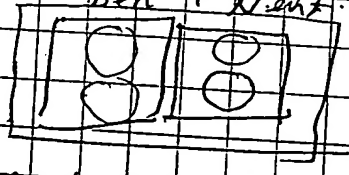


EXHIBIT 12

National Brand

oratory Research

(2) Let anto cross link

(3) Start - 9x roll & Start at 1200 come down to zero.

(4) Dry the blots.

(5) The plates are wrapped in saran wrap and placed at 4°C.

(6) The filters are ~~kept~~ placed in a tray ~~for~~ of H_2O containing ~~2x SSC~~ 2x SSC & 0.2% SDS to remove any protein & debris at 42°C. (≈ 15-30 min.)
(This step not crucial)

(7)

Prehybridization

Soln: Total of 40 ml

20 ml	Formamide
8 ml	SSC 25x
4 ml	Denhardts - (stored at 4°C)
2 ml	$NaPO_4$
4.2 ml	H_2O
2 ml	yeast RNA
0.8 ml	~~2.5%~~ 25% SDS - (add last)

Filter the solution using a 50 ml syringe to a 50 ml tube.

Name: Jagathpala Shah Date: 09/10/99

Experiment: C58 – cloning – contd (Screening of Lebrary)

059

* Open a food bag at one end.
* Take the fetter out using a folded what man and put it to the bottom of the bag.
* Seal ~~the~~ one side of the bag. – 2 seals.
* Pour about 20 ml of the prehyb. solution from front
* Push the air bubbles out carefully. Save 20 ml for hybridization
* Seal the top – 2 seals.
* ~~Pour about 20 ml of the prehybe Sol~~.
* Keep at 42°C – 3 hrs.

~~Purification of~~

Important:

EXHIBIT 14

National® Brand Laboratory Research

Purification of the probe (DNA)

DNA purifying column - ~~cut at the tip~~
Remove the bottom cover. Cut the tip off just below the matrix. Remove the plug off. ~~for~~ Take out the plunger. insert into a 5 ml Syringe.

* ~~Load~~ equilibration of the column :- 5 ml of Elutip-(low salt soln) = ~~Slowly~~ Put the plunger and slowly elute out the equilibration buffer. to a 15 ml tube.
* Take the labelled DNA (crude). Take ≈ 900 ml of Elutip (low salt) :- ~~Elute out the equilibration buffer.~~ Add one more ml of Elutip.
* Put the plunger and slowly press the plunger and get the ~~un~~labelled DNA to a 15 ml tube.
Add ≈ 4 ml of the Elutip to the Syringe (Each time you reload the buffer disconnect the syringe, take the plunger out & then load sample)
* Disconnect the column. Connect to a 2 ml. fresh syringe (take the plunger out before connecting). Load ≈ ½ ml of High salt solution.

EXHIBIT 15

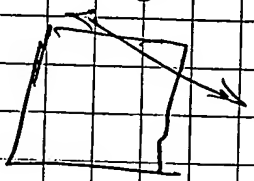
Name: Jeyathpala Sneh Date: 09/10/99

Experiment: C58. Cloning - contd.

Replace the plunger and collect the labelled DNA to a 1ml microfuge tube.

Hybridization.

* Take out the membrane in the bag place a cut across the corner.



* Pour off the soln to sink.
* Take the prefixed probe and boil it for 5 min. (Open the tube in between (after ~40sec) and release press.)
* Take 20ml of the hybridization buffer (saved from earlier prehybridization step) and add the labelled DNA to it.
* Pour this into the bag containing membrane
* Carefully remove the air bubbles out.
* Seal safely - 2
* Get the remaining bubbles to the corner and seal again.
* clean all the area.

Name: Jagathpala Sneha Date: 09/11/99

Experiment: C58- cloning- contd

062

Washing of the membranes:

* Take out the bag, cut the corner - drain it to dead container
* Take out the membranes after cutting 3 sides
* ~~Place the membrane inside~~ Pour 200ml of the washing soln 1.

(1) Washing soln 1:

2X SSC made from 25X SSC) in SDS - 0.2% } 200ml H_2O.

- Pour a small volume pour off after giving a short wash. Pour 200ml of solution (solution at RT) and put the trays at 42°C - 20 min. (It will take time to 42°C by 20 minutes)

(2) Washing Step 2:

0.2% SSC & 0.2% SDS - (200 ml at 42°C)
prewarm the solution to 42°C.
incubate membranes - 20 min

(3) Washing Step 3

200ml of 0.2X SSC & 0.2% SDS - 20 min
pre heat to 50°C (preferably 52°C)
incubate membranes - 20 min

EXHIBIT 17

National Brand Laboratory Research

Name: Jagathpala Sheth Date: 09/12/99.

Experiment: E.58- cloning- Contd.

063

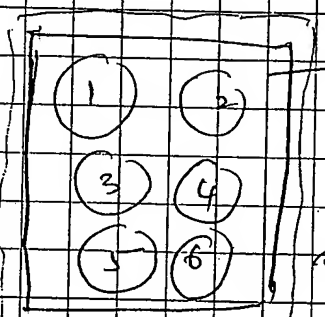
Exposing the membrane

Take membrane in bottle 0·2 SSC and 0·2% SDS.

Take Cassette - mark.

Place the int. screen on a flat surface on the bench place a S. wrap long enough. Place all the membranes in order.

Fold the S. wrap. Place upside down. Fold the sides properly.



Place this on the cassette the marked side up. (Image side is down).

Take small piece of paper containing end-p32. Cut pieces containing one or two dots and paste randomly on

Place one Int. Screen on the top.

Take to the dark room

Place a X-ray film & then another Int. Screen - Put at -70°C

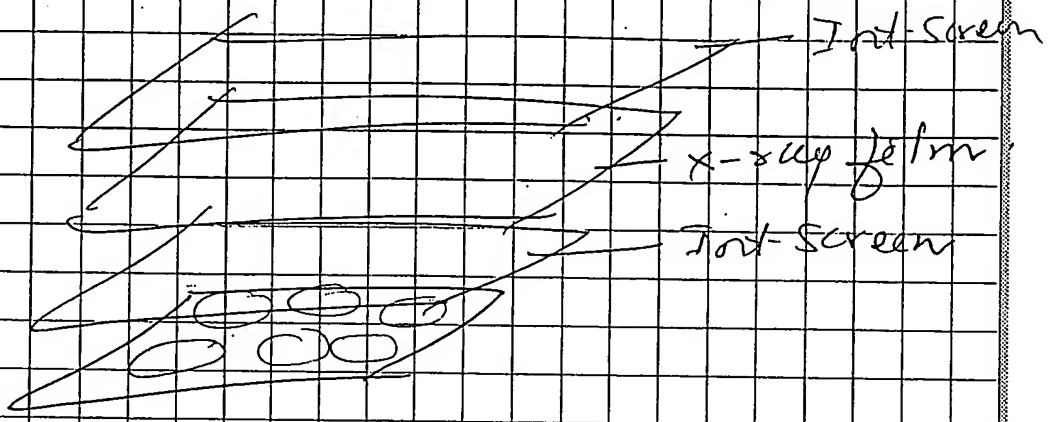
EXHIBIT 18

Name: Jagathpala Shetty Date: 9/11/99

Experiment:

064

9/11/99



9/12/99

Exposed film taken out. One more film put in.

Align the film to the membrane and ~~get~~ to all the marks. Make an impression of the X-ray film. (preferably use different colours for different markings ie: for periphery of the plate, side marks and dot marks inside the membranes.

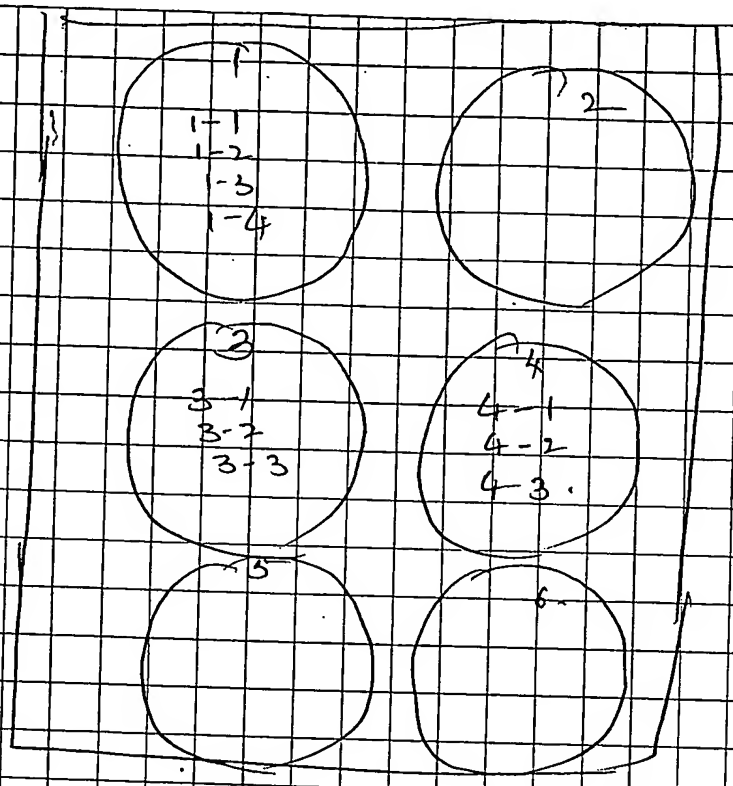
Mark the spots to be picked on the X-ray.

EXHIBIT 19

Name: Jagathpala Shetty Date: 09/13/99

Experiment: C5g - cloning - contd

065



Decide about the spots to be picked.

~~Plate~~ Pipette 0.4 ml of BSM to 10 ml tubes.

Aspirate the spot agar from the plate - shoot positive into tubes containing 0.4 ml of SM.

Put ½ 5% of Chloroform to each tube. (increases in yield + also sterilizes).

↓

put on a vortexing platform at 4°C for about 1-2 hrs

↓

Keep at 4°C till use.

Name: Jagathpala Sheth Date: 09-11-59.

Experiment: C58 cloning - contd.

Secondary Screening

Positive phage - taken out from 4°C

↓

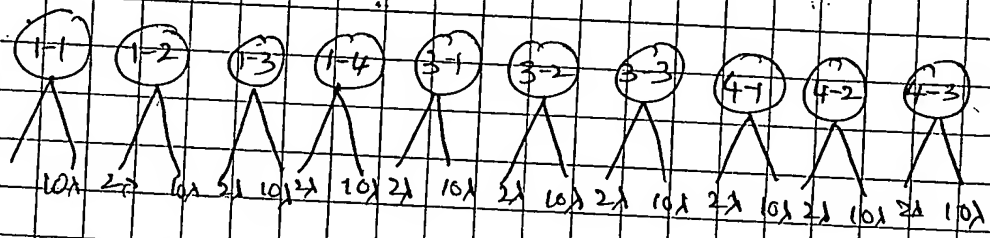
Spin - 2 minutes.

PSM → 90 λ for 10 tubes

18 hr host temp - 90 PSM

↓

Vortex.



Mean time. 20 plates poured - NZCYM agar.

After solidifying, bottom - marked with the ~~numbers~~ corresponding numbers.

NZCYM - agarose - melted - Kept at 50°C.

Taken 2 tubes at a time containing phage - Kept at 50°C for 2 minutes.

↓

Add 4 ml of NZCYM - agarose

↓

poured a top layer on the plates & allowed to solidify

↓

Kept at 37°C

EXHIBIT 21

National Brand

Laboratory Research

Name: Jagathpala Sruthi Date: 09/14/99

Experiment: C58 -- cloning - contd

067

DNA labelling:

50 ng of C58- labeled as before.

9/14

9/15/99

Secondary lifting

Plates taken out from 37°C.

In each pair the plate showing ~200 phage selected

10 nylon (Schleicher & Schuell) - marked

↓

A left was made as before

↓

Denaturation (5-10 Min)

NaOH (0.5M)
NaCl (1.5M)

↓

Neutralization (5-10 min)

0.5M Tris
1.5M NaCl

↓

Cross link

↓

Drop the ~~plates~~ membranes

↓

Wash at 42°C with 2xSSC & 0.2% SDS (30 min)

Name: Jagathpala Shetty Date: 09/05/99

Experiment: C58 cloning - contd.

068

C58

~~The~~ Prehybridization & Hybridization

Membranes

↓

Put in food bag (seal sides)

↓

Pour prehybe solution

↓ 3hrs.

Purify the labelled DNA using elutip. in 50gs

↓

500x of labelled DNA + 4.5 ml of hybe solution

↓

The bag opened & prehybe soln. poured to side

↓

The hybridization done O/N with the label + hybe soln.

EXHIBIT 23

Name: Jagathpala Reddy Date: 09/16/99

Experiment: ESB - Cloning - Contd.

069

Washing of Membranes

① Discarded the hyb. Solution

↓

I Wash 2× SSC, 0.1% SDS — 20 min. 30 → 42°C.
≈ 200 ml

II Wash 0.2× SSC, 0.2% SDS — 20 min. 42°C
≈ 200 ml

III Wash 0.2× SSC, 0.2% SDS — 20 min. 42°C.
≈ 150 ml

↓

Membranes taken in 50 ml of 0.2× SSC & 0.2% SDS.

↓

aligned on the Saran Wrap.

(1-1) (1-2) (1-3) (1-4)
(3-1) (3-2) (3-3)
(4-1) (4-2) (4-3)

exposed at 11-45 AM.

9/17/99

Films developed and marked respective to plates.

EXHIBIT 24

Name: J. Shetty

Date: 09-20-99

Experiment:

070

AM1 cells inoculated in 10ml LB + 1/2 of tetracycline

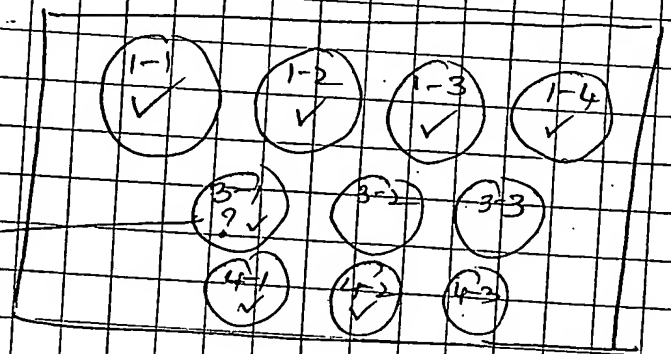
↓ 3 hrs. at 37°C shaker.

Spin the cells

↓

take pellet in 10ml MgSO4 (1mM)

Align the filter on the nitrocellulose and then to the plate - mark the positive clone (isolate) from the back of the plate.



picked one clone each from like picked ones.

↓

in to 1ml tube with 0.5ml of 0.1M

↓

5x of cells/3

↓ 4°C shaker

↓ 1-2 hrs

Give a quick spin to bubble again

↓

30 minutes } Take 15x from the sup

EXHIBIT 25

Name: Jagathpala Sethi Date: 09-20-99

Experiment: C58- cloning- contd.

071

Continued from previous page

↓

Add 50λ of broth (LB) (recombination & circularization)

↓ 1hr. Shaker water bath at 32°C

IPTG 238 mg/ml = 1M

Add 2 µl of 10 mM IPTG to induce replication of recombinant pDR2 plasmids

↓ 1hr. Shaker water bath at 32°C.

Add 1λ of ~~5 µg~~ 500 µg/ml Carbenicillin & 1λ of 1M Sod. citrate (for preferential existence of λDR2 over λDR2)

↓ 32°C for 1 hr.

50λ ≈ 50λ

Spread on LB-Agar plates as follows.

20λ of Carbenicillin 500 µg/ml

45λ of Sod. citrate (1M)

5λ or 50λ of phage λAH1 mix → Spread with Sterile Spreader

↓

o/n 37°C

10/809,654

EXHIBIT 26

National® Brand

ratory Research

Name: Jagathpala Sheth Date: 9/21/99

Experiment: C5B - cloning. Contd.

The plates observed and allowed to grow to larger size at 37°C.

↓

Left at RT for some time.

↓

Inoculation of ~~to~~ to (3 ml) LB cultures

Stock of 50ml LB + 75 µg Amp (50 mg/ml stock of Amp)

made & divided 3 ml each / tube (15 ml tubes)

↓

pick a single isolated colony using tooth pick

choosing any one from a pair

↓

inoculate to LB-Amp.

↓

Shaker - water bath - 37°C O/N.

(?)

(for 3-1 - 2 colonies picked

i.e. 3-1a & 3-1b)

EXHIBIT 27

Research

Name: Jagathpala Sneth Date: 09/22/99

Experiment: Cloning of C58

073

O/N culture of AB1 cells

↓

Qiagen Kit isolation of DNA from plasmid

1. Cells pelleted at 2 steps.

~~(a)~~ - Take 1.5 ml into 1.5 tube - Spin (1½ min) discard supernatant, add another 1.5 ml and take the supernatant using Vacuum-dispenser.

Follow the Qiagen kit protocol to isolate DNA

① ~~Dislodge pellet~~ Add 0.3 ml of BF P1 dislodge pellet using P 200 pipetteman

② Add 0.3 ml of BF P2 - invert 4-6 ~~bottle~~ times - Sit 5 minutes. → bottle at 4°C

③ Add 0.3 ml of P3 - ~~Stagently~~ invert 4-6 times - keep ~~on~~ ice - 5 minutes & spin - 10 minutes

④ Clean while set up the Qiagen column.

equilibrate the column with 1 ml of QBT

Important

Name: Jagathpala Sneh Date: 09-22-99

Experiment: cloning of C58

074

(5) Take the supernatent carefully from step (3) leaving the upper layer and the lower viscous pellet and load to the column carefully.

(6) Wash the column with 1ml x 4 (times) of solution QC. Wait till last drop. Put the tube at the bottom of column

(7) Elute DNA with 0.8 ml of QF Wait till last drop

(8) Discard the column

(9) Add 0.56 ml of isopropanol

(10) Spin for 30 minutes, 12,000 rpm

(11) Take sup. with fine tipped pasteur pipette with a bulb. (Create one fine tipped pasteur pipette)

(12) ~~Give~~ a carefully load 200 λ of chilled 70% ethanol and once again take the sup. off.

(* DO not disturb the pellet)

↓

Air dry.

Important: --

10/809,654

EXHIBIT 29

Laboratory Research

National Brand

Name: Jegathpala Shetty Date: 9-22-99

Experiment: Cloning of C58

075

Dissolve DNA in 20 λ of Sterile water

Keep on shaker at 4°C - 15 min

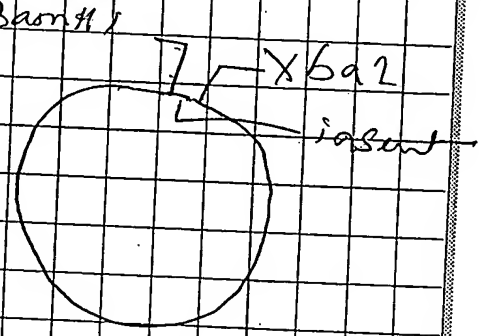
↓

Shake again at ~ 20°C bath thermomixer - 3-5 minutes

↓

Give a quick spin.

Digestion of plasmid with BamH1 and XbaI



(Usually buffer conditions are different for 2 enzymes)

BamH1 (Roche)

XbaI (Roche) In this case same buffer used.

For 500 λ tube

Vortex - give a quick spin	DNA	- 20 λ
	BSA	0.5 λ
	10× BamH1 Buf	0.5 λ
	XbaI	1.0 λ
Add last mix thoroughly & Quick spin	BamH1	0.5 λ

EXHIBIT 30

National Brand

boratory Research

Name: J. Shetty Date: 9/23/99

Experiment: Restriction digestion of DNA (plasmid)

077

Digestion of DNA - Sequential digestion

Cocktail for Xba I

9 λ of	10 x Bf
9 λ of	1 mg/ml BSA
(41.5) λ of	H_2O
9 λ of	Xba I

Add these, cool & then add enzyme.

prepared for 18 reactions

Xba I

Taken 1 λ of DNA + 4 λ of cocktail mixed with pipette tip

↓

37°C - 4.5 hrs

Bam-HI

Cocktail for Bam HI

18 λ	5M NaCl - to bring the desired conc to ~ 200 mM (?)
9 λ	10X Bam HI Bf
9 λ	Bam HI
9 λ	1 mg/ml BSA
61.2 λ	H_2O

prepared for 18 reactions

Added 5 λ each to tubes

= 37°C O/N

Important

EXHIBIT 21

National Brand Laboratory Research

Name: J. Shetty

Experiment: Agarose gel electrophoresis of digested DNA   Date: 9/24/99

1.2% Agarose Gels

Lanes:

1. 1-1 → M
2. 1-2 — showed around 1 Kb DNA band
3. 1-3
4. 1-4 → M
5. 3-1a — showed around 900 b pair product
6. 3-1b — "
7. 4-1
8. 6-2 — showed around 1 Kb product. → M

(1-2) and (6-2) — Given for Sequencing

DNA - 3λ

Forward primer } 1.5λ - 23 mer

11.5 → 16 λ

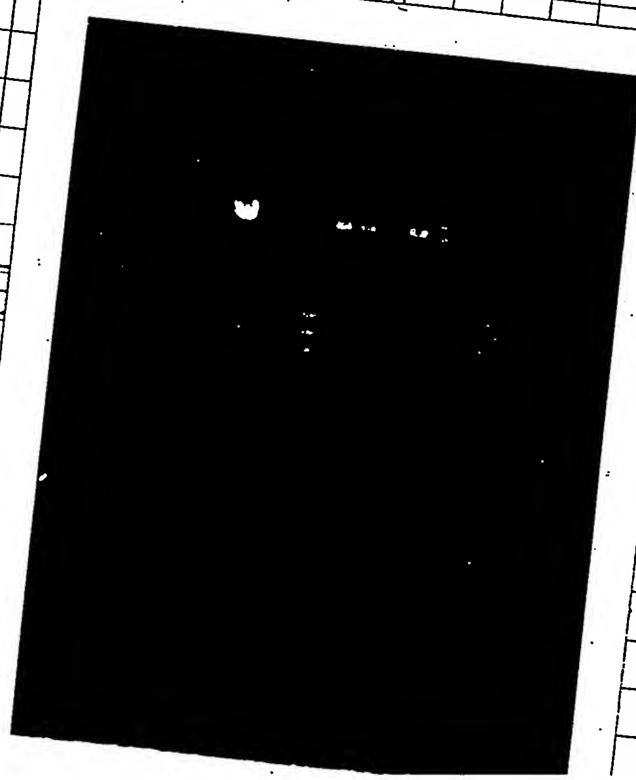
DNA

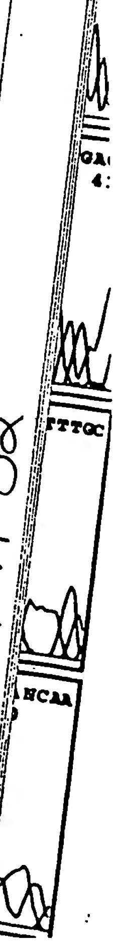
Rev primer } 2.5λ - 21 mer

H_2O 11.5λ

16λ

1.078 ←
872 ←
603 ←





Brand Research

EXHIBIT 32

Got the Sequence back

Sequence - bad - ~~as~~ -

- Decided to give more DNA for now 1-2r

DNA : 11.5 λ
For. prim : 1.5 λ
H_2O 30 λ
16 λ

A culture of bacterial cells - with clone - (1-2) and (4-2) (saved earlier) - inoculated to 2L 2S and culture - with amp. and Sod. Citrate. (10 mM) (75 μg/mL)

O/N

Name: J. Shetty Date: 9/25/99

Experiment: Plasmid isolation

080

Alkali preparation of plasmid DNA

25ml culture

↓

poured into 2 - 15 ml tubes

↓ spin - 3000 rpm.

~~Pellet~~ Supernatant discard completely

↓

pellet

↓

processed for DNA isolation using Qiagen kit

↓

pellet obtained at the final step - ~~carefully~~ carefully washed ~~with~~ ~~add~~ with 2ml of EtOH - ~~70%~~ (chilled)

↓

pellet dried completely

↓

resuspended in 80µl of d.H2O.

↓

Saved in -70°C.

EXHIBIT 34

Name: Jagathpala Shetty Date: 10/5/99

Experiment: Sequence for C58 / 1-2F

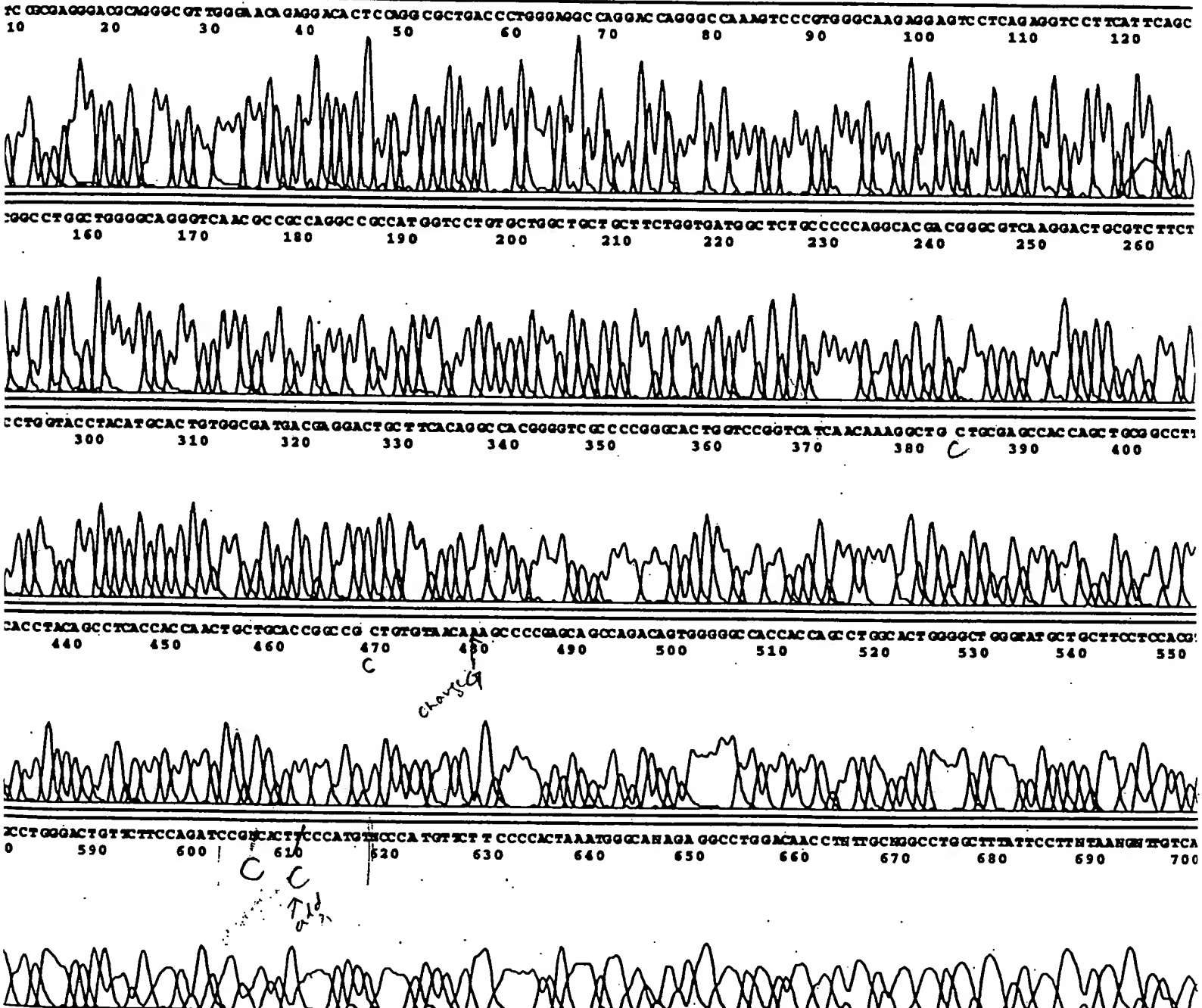
082

Sequence for 1-2F - Obtained -

17-99-13259
1-2 F
99-13259
Lane 17

Signal G:402 A:243 T:156 C:338
DT {BD Set Any-Primer}
dRmatrix61697
Points 938 to 10624 Pk 1 Loc: 938

Pag
Tue, Oct 5, 1999
Mon, Oct 4, 1999
Spacing: 8.9



Important: Place card under blue copy.

EXHIBIT 35

Name: Jagathpala Shelly Date: 10/6/91

Experiment: Sequence for C58

083

Sequence for 1-2 R & 4-2 R obtained.
However sequences were bad.
They were resubmitted with a
request for]>(dt)?]>(dT) 20N
primer

However the sequence results of
clone 1-2 F yielded enough
(good) Sequence to deduce the complete
Open reading frame for C58 !

Important

EXHIBIT 2/0

Nucleotide and deduced amino acid sequence for C58

Complete ORF of C58 contained 372 base pairs encoding 124 amino acids with a predicted Mol Wt. of 13 and a predicted pI of 5.5. Sequences of one of the tryptic peptides originating from the cored 2-D spot was found embedded in the ORF (Blue boxes).

```
GTCCCGGATCCGCGAGGGACGCAGGGCGTTGGGAACAGAGGACACTCCAGGCGCTGACCC
V  P  D  P  R  G  T  Q  G  V  G  N  R  G  H  S  R  R  *  P  -
TGGGAGGCCAGGACCAGGGCCAAAGTCCCGTGGGCAAGAGGAGTCCTCAGAGGTCCTTCA
W  E  A  R  T  R  A  K  V  P  W  A  R  G  V  L  R  G  P  S  -
TTCAGCGGTTCCGGGAGGTCTGGGAAGCCCACGGCCTGGCTGGGGCAGGGTCAACGCCGC
F  S  G  S  G  R  S  G  K  P  T  A  W  L  G  Q  G  Q  R  R  -
CAGGCCGCCATGGTCCTGTGCTGGCTGCTGCTTCTGGTGATGGCTCTGCCCCCAGGCACG
Q  A  A  M  V  L  C  W  L  L  L  L  V  M  A  L  P  P  G  T  -
                1
ACGGGCGTCAAGGACTGCGTCTTCTGTGAGCTCACCGACTCCATGCAGTGTCCTGGTACC
T  G  V  K  D  C  V  F  C  E  L  T  D  S  M  Q  C  P  G  T  -
TACATGCACTGTGGCGATGACGAGGACTGCTTCACAGGCCACGGGGTCGCCCCGGGCACT
Y  M  H  C  G  D  D  E  D  C  F  T  G  H  G  V  A  P  G  T  -
GGTCCGGTCATCAACAAAGGCTGCCTGCGAGCCACCAGCTGCGGCCTTGAGGAACCCGTC
G  P  V  I  N  K  G  C  L  R  A  T  S  C  G  L  E  E  P  V  -
AGCTACAGGGGCGTCACCTACAGCCTCACCACCAACTGCTGCACCGGCCGCCTGTGTAAC
S  Y  R  G  V  T  Y  S  L  T  T  N  C  C  T  G  R  L  C  N  -
AGAGCCCCGAGCAGCCAGACAGTGGGGGCCACCACCAGCCTGGCACTGGGGCTGGGTATG
R  A  P  S  S  Q  T  V  G  A  T  T  S  L  A  L  G  L  G  M  -
CTGCTTCCTCCACGTTTGCTGTGACCAACAGGGAGGACAGGGCCTGGGACTGTTCTTCCA
L  L  P  P  R  L  L  124
                   P  T  G  R  T  G  P  G  T  V  L  P  -
```

Name: Jagathpala Shelly Date: 2/10/99

Experiment: Recombinant expression of C58

Primers ordered for the generation of C58 - ORF - DNA - both Xho and NCO site on either side to be ligated to a PET 20 vector

Name: Jagathpati Sheth Date: 11/2/99

Experiment: PCR to generate C58-complete ORF

086

PCR reaction

Use C58 PET primers.

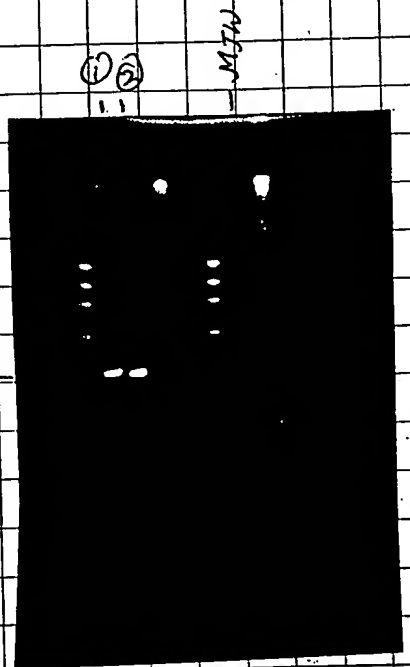
Bottom	~~Top~~	Top
3.025	3.3 dH	4.55
2	4dNTP	
2	Mg	
1.25 (20pM/λ)	~~GSP F~~ (C58PETF)	
1.25 (20pM/λ)	GSP R (C58PETR)	
0.425	H2O	7.95
	cDNA	2
	polymerase	0.5

① C58PET-R-60pM/λ

② C58PETR-20pM/λ

PCR programme (J54)

①	94°C	2:00	
②	94°C	:30	
③	72°C	2:30	11 times
	~~Δ~~ -1°/cycle		
④	Go to ②		
⑤	94°	:30	
⑥	60°	:30	27×
⑦	72°	2:00	
⑧	Go to	5	
⑨	72	18:00	
⑩	4°C	∞	
⑪	END		



Result: Gave the expected size product

Important: Place card under blue copy.

EXHIBIT 39

Name: Jagathpala Sheth Date: 11/16/99

Experiment:

089

Digestion of C58-RET-DNA with Xho I and NCo-I endonucleases.

DNA received in 90λ → Dato evaporated to ≈ 15λ.

Digested with Xho I & NCo I as follows:

	DNA	15λ
(promega) bf	D (10x)	2.5λ
	~~Xho~~ H_2O	3.5λ
Boringer	Xho I	2λ
NEB.	NCo I	2λ
		25λ

→ 37°C O/N water bath.

11/17

Purification of DNA by gel electrophoresis

Loaded all 25λ + 4λ of loading bf.

Used 5 wells (combed with tape) / 5 wells

DNA purified received in ≈ 80λ of H_2O

↓

Desalted using Centricon X2 tubes

↓

received in 60λ

↓

Quantified.

Important: —

EXHIBIT 40

National Brand Laboratory Research

Name: Jagathpala Sheth Date: 11/17/99

Experiment:

090

Samples: ① 5λ of DNA + 1λ of loading dy.
② 3λ of DNA + 2λ "
③ 1λ of DNA + 4λ "
④ 0.5λ of marker
⑤ 1.0λ of marker
⑥ 1.5λ of marker

Actual amount of DNA: $125 \times 1 \times \frac{0.603}{5.386} \times 1$

$= 13.99$ ng/λ

Total vol: 50λ. i.e.: 699.75 ng

Name: Jagathpala Shetty Date: 11/18/99

Experiment:

Ligation

NCO/Xho cut	pET C58 -	3λ	
	pET 28b+	2λ	
	10X lig bf	2λ	(also contain ATP)
	H2O	12.5 λ	
	ligase	0.5 λ	
		20 λ	

↓ 14°C O/N

After putting DNA, H2O & vector ~~warmed~~ to ~50°C in water bath ~~and~~ for 30 secs. ~~and~~ and cooled at RT.
Then added ~~bf~~ lig-bf mix thoroughly and gently add ligase on ice.

Culture of host strain bacteria – Nova Blue. / ~~DE3~~ BL-21

15ml of LB + spec of strain – O/N 37°C.

11/19/99

Preparation of Competent cells and transformation of DNA to host strains

1. Culture diluted 5 times and checked O.D (BL-21) = 0.8
   Novablue = 0.8
2. Diluted the culture back down to

0·1 OD in 1·25 ml LB + 12·5 µl 1M $MgCl_2$/SO_4

ie: 170 µl ~~+ 25~~ of culture used.

↓

Grown to ~ 0·55 OD at 37°C Shaking

↓

Centrifuged remove supernatant

↓

Redissolved in 0·4 ml TFB (from MJW)
and Keep in ice - 10'

↓

Centrifuged, dissolve - 100 µl of TFB
Add 3·5 µl DMSO (from MJW)
Keep in ice 10'
Add 3·5 µl DMSO again
Keep in ice 10'

↓

Added 10 µl each of ligation mixture
& kept in ice - 30'

↓

Given a heat Shock @ 42°C for 90sec.

↓

Kept in ice 2'

↓

Added 300 µl LB + $MgCl_2$ (3 µl) SO_4 + Glucose (20mM) (ie: 8 µl from MJ)

↓

Shaken at 37°C - 1 Hr.

EXHIBIT 43

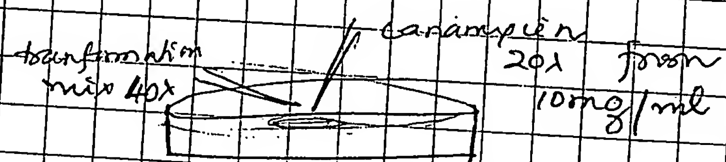
Name: Jagathpala Sheth Date: 11/19/99

Experiment:

Plating.

Plating was done on LB-agar plates.

From each tube 3 plates were plated (from for BL-21 & another each) at 40λ, 360λ & 45λ neat 10μg/ml of canamycin as the selection.



↓

Spread.

↓

37°C O/N.

11/20/99. One colony picked from plates 40λ ① & 360λ ② from each strain and a 3ml O/N culture made in LB + 1% glucose + canamycin 10μg/ml.

EXHIBIT 44

Name: Jagathpala Shetty Date: 11/22/99.

Experiment: Plasmid isolation

Isolation of Plasmid DNA

Isolation made by following the protocol for the Qiagen kit for minipreps.

(1) 3ml of culture centrifuged in 1.5ml microfuge tubes at 2 steps.

↓

(2) Add 0.3ml of bf. P1 to the pellet dislodge the pellet with P200 micropipette.

↓

(3) Add 0.3ml of bf P2 - invert 4-6 times at 4°C - sit - 5 min

↓

(4) Take P3 from 4°C and add 0.3ml to tubes and invert 4-6 times and place it on ice - 5 min and

↓

Spin 10 min

(5) Mean while set up the Qiagen column. Equilibrate the column with 1ml of QBT

↓

Take the supernatant from step 4 carefully and load to the column.

↓

(6) Wash the column with 1ml x 4 of QC. Wait till last drop drops off.

Name: Jagathpala Shetty

Experiment:

Date: 11/21/99

(7) Elute DNA in 0.8 ml of QF
wash till the last drop

↓

(8) Add 0.52 ml of isopropanol
to

(9) Spin for 30 minutes at 10,000 rpm

↓

(10) Remove sup. with fine tipped
pasteur pipette

(11) Carefully coast the pellet with
200 l of chilled 70% ethanol.

↓

(12) Air dry.

11/21/99

Resuspend the DNA in 20 l
each of sterile water.
mix at 4°C for 15-20 min

EXHIBIT 46

Name: Jayathpala 'shetty' Date: 11/22/99.

Experiment:

Digestion of plasmid DNA with XhoI and NCoI.

H_2O	
DNA	2.5 λ
(Boerenga) XhoI	1 λ
(NBiolabs) NCoI	1 λ
(Promega) BSA (10x)	0.5 λ
	5 λ

} 37°C O/N

11/23/99 2% agarose gel electrophoresis of digested DNA

① Marker ϕ
② BL-21 - ①
③ BL 21 - ②
④ NOV BL ①
⑤ NOV BL ②
⑥ Marker λ Hind III
⑦ Marker ϕ

clone #④ (NOV-BL-②) gave the right size insert.
A Glycerol stock of the same - done

EXHIBIT 47

Name: Jagathpal a Shetty Date: 11-2.-99

Experiment: Sequencing of the vector.

DNA from clone #4. Nara. Blue - ② - was given for sequencing.

① DNA : 8 µl

T7 terminator : 2 µl (5 pmoles/µl)

H_2O 6 µl

16 µl

② DNA : 8 µl

T7 promoter — requested from Bank

H_2O 4 µl

12 µl

Important: Place card under blue copy. EXHIBIT 48

Name: Tagathpha Shetty Date: 11/23/05

Experiment: Sequence of c58 in pET 28 b after ligation 098

(Linear) MAP of: petc58.promoter.dna check: 7309 from: 1 to: 663

DNA sequence of pET28b-c58.novablue. with T7 promoter as the primer transformed on 11-19-99.

With 2 enzymes: NCOI XHOI

November 29, 1999 14:35 ..

```
                                                                NcoI
          GGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGG
        1 ---------+---------+---------+---------+---------+---------+ 60
          CCTATTGTTAAGGGGAGATCTTTATTAAAACAAATTGAAATTCTTCCTCTATATGGTACC
  c         I  T  I  P  L  *  K  *  F  C  L  T  L  R  R  R  Y  T  M  V -

          TCCTGTGCTGGCTGCTGCTTCTGGTGATGGCTCTGCCCCCAGGCACGACGGGCGTCAAGG
       61 ---------+---------+---------+---------+---------+---------+ 120
          AGGACACGACCGACGACGAAGACCACTACCGAGACGGGGGTCCGTGCTGCCCGCAGTTCC
  c         L  C  W  L  L  L  L  V  M  A  L  P  P  G  T  T  G  V  K  D -

          ACTGCGTCTTCTGTGAGCTCACCGACTCCATGCAGTGTCCTGGTACCTACATGCACTGTG
      121 ---------+---------+---------+---------+---------+---------+ 180
          TGACGCAGAAGACACTCGAGTGGCTGAGGTACGTCACAGGACCATGGATGTACGTGACAC
  c         C  V  F  C  E  L  T  D  S  M  Q  C  P  G  T  Y  M  H  C  G -

          GCGATGACGAGGACTGCTTCACAGGCCACGGGGTCGCCCCGGGCACTGGTCCGGTCATCA
      181 ---------+---------+---------+---------+---------+---------+ 240
          CGCTACTGCTCCTGACGAAGTGTCCGGTGCCCCAGCGGGGCCCGTGACCAGGCCAGTAGT
  c         D  D  E  D  C  F  T  G  H  G  V  A  P  G  T  G  P  V  I  N -

          ACAAAGGCTGCCTGCGAGCCACCAGCTGCGGCCTTGAGGAACCCGTCAGCTACAGGGGCG
      241 ---------+---------+---------+---------+---------+---------+ 300
          TGTTTCCGACGGACGCTCGGTGGTCGACGCCGGAACTCCTTGGGCAGTCGATGTCCCCGC
  c         K  G  C  L  R  A  T  S  C  G  L  E  E  P  V  S  Y  R  G  V -

          TCACCTACAGCCTCACCACCAACTGCTGCACCGGCCGCCTGTGTAACAGAGCCCCGAGCA
      301 ---------+---------+---------+---------+---------+---------+ 360
          AGTGGATGTCGGAGTGGTGGTTGACGACGTGGCCGGCGGACACATTGTCTCGGGGCTCGT
  c         T  Y  S  L  T  T  N  C  C  T  G  R  L  C  N  R  A  P  S  S -

          GCCAGACAGTGGGGGCCACCACCAGCCTGGCACTGGGGCTGGGTATGCTGCTTCCTCCAC
      361 ---------+---------+---------+---------+---------+---------+ 420
          CGGTCTGTCACCCCCGGTGGTGGTCGGACCGTGACCCCGACCCATACGACGAAGGAGGTG
  c         Q  T  V  G  A  T  T  S  L  A  L  G  L  G  M  L  L  P  P  R -

                XhoI
                 |
          GTTTGCTGCTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAA
      421 ---------+---------+---------+---------+---------+---------+ 480
          CAAACGACGAGCTCGTGGTGGTGGTGGTGGTGACTCTAGGCCGACGATTGTTTCGGGCTT
  c         L  L  L  E  H  H  H  H  H  H  *  D  P  A  A  N  K  A  R  K -

          AGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCT
      481 ---------+---------+---------+---------+---------+---------+ 540
          TCCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGGGGAACCCCGGA
  c         E  A  E  L  A  A  A  T  A  E  Q  *  L  A  *  P  L  G  A  S -

          CTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATG
      541 ---------+---------+---------+---------+---------+---------+ 600
          GATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCCTTGATATAGGCCTAACCGCTTAC
  c         K  R  V  L  R  G  F  L  L  K  G  G  T  I  S  G  L  A  N  G -
```

C58- is Successfully ligated to the pET28b Vector!

10/809,654

EXHIBIT 19

Name: Jagathpala Shetty Date: 11-25-97

Experiment:

A O/N culture from pET 28b-C58-NovaBlue (#4) ~~tube~~ was made in tubes (3 ml each)

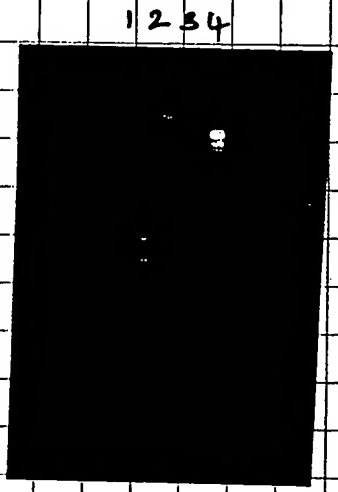
↓

plasmid DNA isolated.

11/26/97

11/29/97 A 2% agarose gel ran.

① Marker
② Tube #1 from pET 28b-C58-NovaBlue #4
③ Tube #2 from " " "
④ Marker



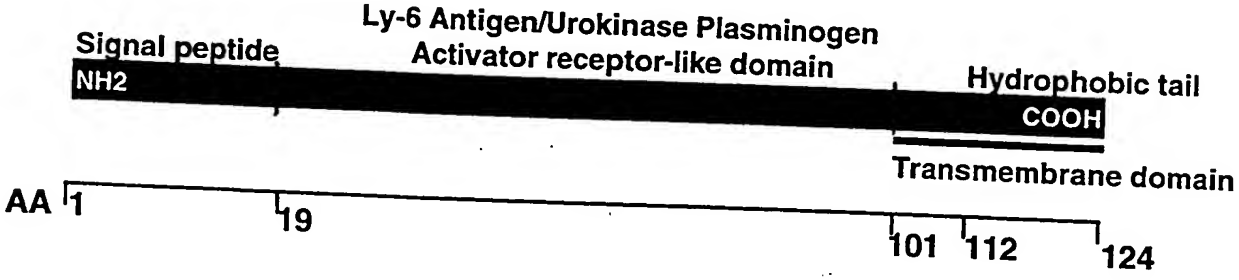
The host strain bearing the plasmid gone bad. 07 was denoted ↓

Streak a plate, pick a single colony and make a glycerol stock of the construct.

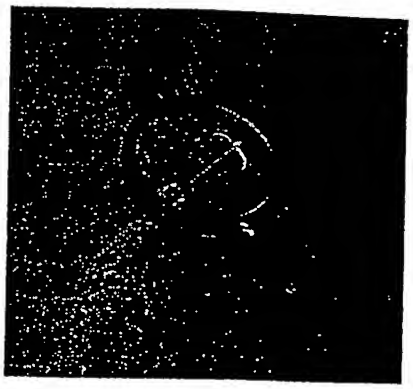
Important: Place card under blue cover.

EXHIBIT 50

Fig. 8. Proposed Architecture of C58



C58 is GPI anchored - It has a sig. pept. a C-terminal transmembrane domain & transamidase cleavage site!



N-terminal signal peptide
1 19
MVLCWLLLLVMALPPGTTGVKDCVFCELTDSMQCPGTYMHCGDDEDCFTGHGVAPGTGPVINK

97
GCLRATSCGLEEPVSYRGVTYSLTTNCCTGRLCNRAPSSQTVGATTSLALGLGMLLPPRLL

Transamidase C-Terminal Transmembrane domain

EXHIBIT 51

Sequence alignment of C58 with other Ly6/uPAR family members.

```
C58          (24- 98):VFCELTDSMQCPGTYMHCGDDEDCFTGHGVAPGTGPVIN---KGCLRATSCGLEEPVSYRGYTYSLTTNCCTGRLCNRA
CD59-AOTTR (12-126):CPYPTTQ---CTMTTNCTSNLDSCLIAKA-GSRVYYR-------CWKFEDCTFSRYSNQLSEN-ELKYYCCKKNLCNFN
CD59-CALSQ (12-126):CPYSTAR---CTTTTNCTSNLDSCLIAKA-GLRVYYR-------CWKFEDCTFRQLSNQLSEN-ELKYHCCRENLCNFN
CD59-SAISC (12-128):CPLPTMESMECTASTNCTSNLDSCLIAKA-GSGVYYR-------CWKFDDCSFKRISNQLSET-QLKYHCCKKNLCNVK
CD59-CERAE (12-126):CPNPTTD---CKTAINCSSGFDTCLIARA-GLQVYNQ-------CWKFANCNFNDISTLLKES-ELQYFCCKKDLCNFN
CD59-PAPSP (12-124):CPNPTTN---CKTAINCSSGFDTCLIARA-GLQVYNK-------CWKFEHCNFNDVTTRLREN-ELTYYCCKKDLCNFN
CD59-HUMAN (12-126):CPNPTAD---CKTAVNCSSDFDACLITKA-GLQVYNK-------CWKFKKCSFKRISNQLSET-QLKYHCCKKNLCNVN
CD59-HSVSA  (7-117):CSHSTMQ---CTTSTSCTSNLDSCLIAKA-GSGVYYR-------CWRFDECNFDFISRNLAEK-KLKYNCCRKDLCNKS
CD59-PIG   (12-123):CINPAGS---CTTAMNCSHNQDACIFVEAVPPKTYYQ-------CWRFSDCNAKFILSRLEIA-NVQYRCCQADLCNKS
CD59-RAT:   (9-120):CLDPV-SS--CKTNSTCSPNLDACLVAVS-GKQVYQQ-------CWRFSDCNAKFILSRLEIA-NVQYRCCQEDLCNV-
LYGA-MOUSE  (2-134):CYGVPFET-SCP-SITCPYPDAVCISQQVELIVDSQRRKVKNKLCFPFCPANLENMEILGTTV-NVNTSCCKEDLCNA-
LYGF-MOUSE (11-107):CLGVSLGI-ACK-SITCPYPDAVCISQQVELIVDSQRRKVKNKLCFPFCPANLENMEILGTTV-NVNTSCCKEDLCNA-
LYGC-MOUSE  (2-131):CYGVPIET-SCP-AVTCRASDGFCIAQNIELIEDSQRRKLKTRQCLSFCPAGVP---IKDPNI-RERTSCCSEDLCNA-
LYGE-MOUSE (11-107):CTDQKNNI-NCLWPYSCQEKDHYCITLSAAAGFGN-YNLGYTLNKGCSPICPSENYNLNLGYA-SYNSYCCQSSFCNFS
E48A-HUMAN (21-93) :CTSSSN----CKHSYYCPASSRFCKTTNTYEPLRGNLYK----KECANSCTSDYSQQGHYSSG-SEVTQCCQTDLCNER
THYB-MOUSE (3-117) :CTNSAN----CKNPQYCPSNFYFCKTYTSYEPLNGNLYR----KECANSCTSDYSQQGHYSSG-SEVTQCCQTDLCNER
UPAR-RAT  (17-132) :CESNQD--CLYEECALGQ---DLCRTTYLREWEDAEELEYYTRGLCHKEKTNRTMSYRMGSYIYSLTETYCATNLCNRP
UPAR-MOUSE(14-131) :CESNQS--CLYEECALGQ---DLCRTTYLREWQDDRELEYYTRGCAHSEKTNRTMSYRMGSMIISLTETYCATNLCNRP
UPAR-HUMAN(14-129) :CKTNGD--CRYEECALGQ---DLCRTTIYRLWEEGEELELYEKSCTHSEKTNRTLSYRTGLKITSLTEYYCGLDLCNQG
UPAR-BOVIN (5-127) ;CENTTS---CSYEECTPGQ---DLCRTTYLSYWEGGNEMNYYRKGCTHPDKTNRSMSYRAADQIITLSETYCRSDLCNKP
```

Name: Jagathpi in Sheth Date: 11/29-99.

Experiment: Bacterial expression of C58

01

Bacterial cells (NOVA BLUE) containing the construct pET 28b - C58 (#4) was streaked on a agar plate (LB)

11/30/

picked a single colony and inoculated to 1ml LB broth

↓

A glycerol stock made (1ml of culture + 150λ of 100% glycerol)

Protein Expression

10λ of the culture from above taken - inoculated to 2ml LB culture medium + kanamycin 10μg/ml

↓

grown to ~ 0.5 OD

~~cool~~ at ↓

4°C O/N

↓

12/1/99

cultures from above inoculated to 20ml culture (LB + Kanamycin)

Important: Place card under blue copy. EXHIBIT 53

Name: Jagadeesha Shetty Date: 1/1/99

Experiment:

0

Expression - Continued

Expt.

20 ml culture

↓

0.7 OD

↓

induced with 1 mM IPTG

Stock 200 mg/ml (840 mM)

4 samples with 0.5 O.D/ml saved) at 0 time of induction)

↓

O.D after 2 hrs - 2.

Sample collected after 2 hrs after induction.

(0.5 O.D/ml = 4 samples)

↓

O.D after 3 hrs - 2.8 -

4 0.5 O.D/ml samples saved

↓

Kept on ice.

↓

Centrifuge

↓

Save pellet at -20°C

Control

20 ml culture.

↓

0.7 OD

not induced

0.5 O.D/ml samples (4) saved

↓

sample collected after 2 hrs

0.5 O.D/ml = 4 samples

↓

3 hrs O.D = 2.8

4 0.5 O.D samples saved

↓

Kept on ice

↓

Centrifuge

Save pellet at -20°C

Name: Jagadhyala Sneha Date: 12/3/19

Experiment:

03

Bacterial lysate preparation and electrophoresis

① total cell preparation

② soluble fraction

③ insoluble fraction

① Total cell: 0.5 OD pellet + 20% of ~~0.5 M~~ 10 mM Tris, pH 8.0 + 20% of sample buffer

Boil 70°C – 2 min

centrifuge

load everything.

② preparation of soluble & insoluble fractions:

used Bugbuster – novagen

0.5 OD/ml pellet

add 95% of bugbuster

vortex

shaker 10 min

centrifuge

pellet ← supernatant → soluble fraction

add bugbuster 20%

mild vortex

Add 200 kg/ml lysozyme

incubate 5 min

Sample buffer

load

Name: Jagatha la Shetty Date: 12/1/09

Experiment:

Insoluble fraction - continued.

↓

Add 6 vols of 1:10 bugbuster

↓ vortex.

centrifuge

↓

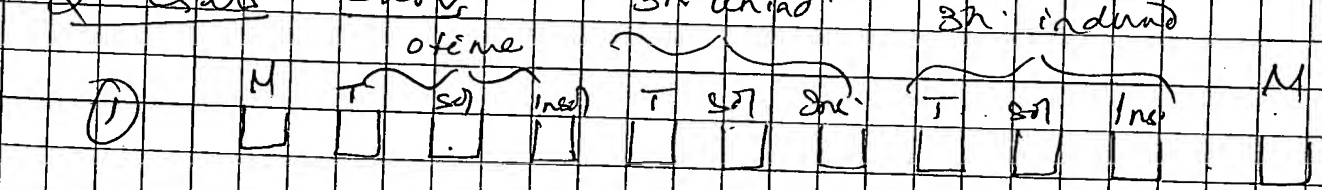
pellet + 1:10 bugbuster

↓ centrifuge

pellet resuspend in

10 mM Tris + Sample buffer.

2 Gels run.



15% separating gel 4% Stacking gel

run O/N at 15 mAmps

↓

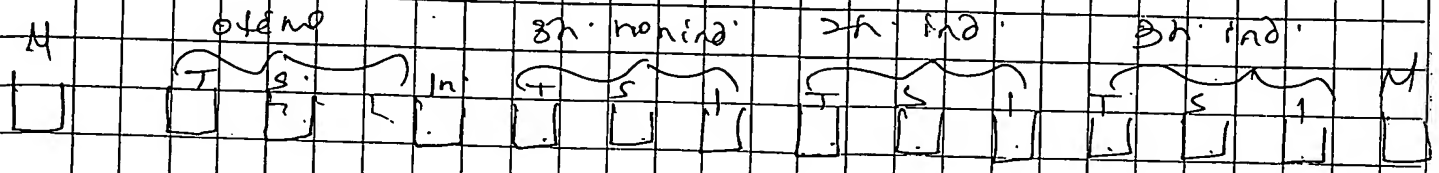
Gel coomassie Stained.

Name: Jagathg. Na Sheth Date: 1/4/99

Experiment:

05

Gel #2.



Gel transfered to a nitrocellulose membrane

12/6/99

Western blotting of the membrane

used 1:1000 dilution of Ni-NTA conjugate

↓

developed by ECL + W.B.

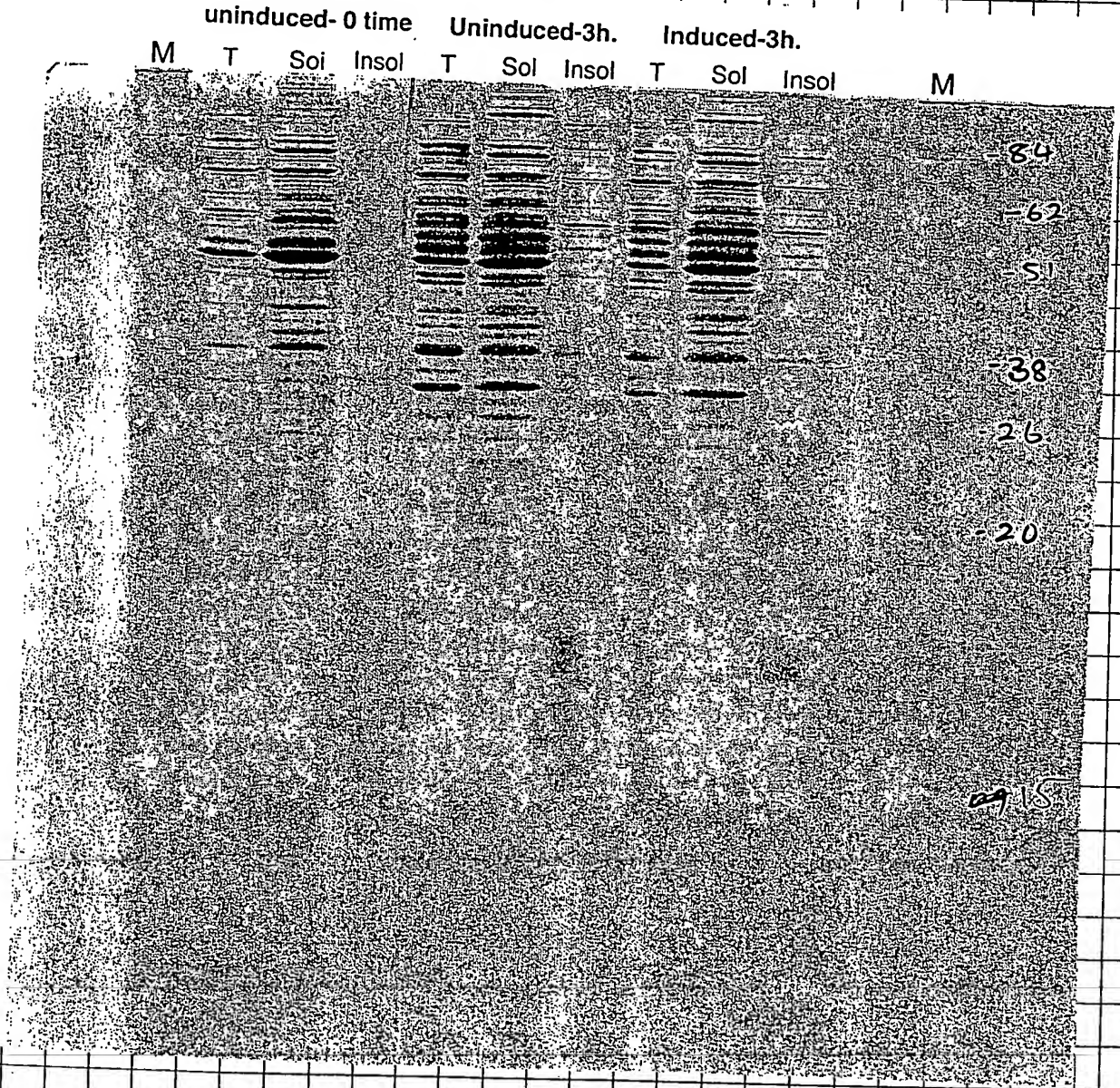
Blot was pretained with pressure S. before probing.

Name: Jagathy La Shetty Date: 12/1/99

Experiment:

06

Gel # 1 : Coomassie stained Gel



No expression of

Important: Place card under blue copy. EXHIBIT 58.

Name: Jagathpal Shetty Date: 12/1/99

Experiment: C58 - Rec. expression

09

(1) Nova blue - pET 28b - control - 7.6ng/λ

(2) BL-21 - pET 28b - control - 1.15λ from stock - 7.6ng/λ

(3) BL 21 - C-58 - 28b - transformed with C-58 plasmid DNA - 1λ

Plated 360λ & 40λ

at 37°C o/n.

12/11/99 Plates examined and kept at 4°C

12/12/99

Single colony from one of the plates from each group - inoculated - 1ml o/n culture made

↓

12/13/99 (1) Glycerol stock of all the 3 made.

(2) A 2ml culture for pET 28b-C58 & pET 28b - (control) made.

left the OD reached 4.005

↓

kept at 4°C o/n

Important: Place card under blue copy. EXHIBIT 59

Name: Jagathpala Shetty Date: 12/13/19

Experiment: C58- Rec! expression

Inoculated 3 ml of innoculum from control (empty vector) and C58+ vector-Novable to 20 ml culture.

↓

At O.D = 0.7 added 1mM IPTG to the culture

Sample saved before induction (0.5 of 0.5/ml samples)

↓

After 2 hrs. - samples saved (0.5 OD samples)

↓

After 3 hrs. flasks taken out chilled - ice.

↓

0.5 OD samples - aliquoted - centrifuged

Rest of the samples - centrifuged and saved.

↓

pellets saved at -20°C

SDS- PAGE of the ~~culture~~ bacterial lysate

Gel : 15%.

Sample preparation :

used bug buster - pellets dissolved in 30 λ of bug buster - vortexed - 5 min. centrifuged → Sup + 30 λ of sample & 90°C 2 min - load

pellet + 30 λ bug buster

↓ vortex.

+ lysozyme 200 µg/ml

↓ incubate 5 min.

Add 180 λ of 1:10 bug buster

↓ Vortex

↓ Centrifuge 4°C 20 min

pellet → add 200 λ of 1:10 bug buster

↓ vortex

pellet → add 200 λ of 1:10 bug buster

↓

pellet + ~~add~~ resuspend in Tris buffer 50 mM

Add eq. volume of sample buffer & to

heat to 90°C 2 min

load

Name: Jagathpal Shetty Date: 12/1.99

Experiment:

Controls: 200 µg of lysozyme in 50 mM Tris and Sample buffer, & sets of gels run.

Coomassie Staining

Transfer to nitrocellulose & probed with Ni-NTA (1:2000).

TMB

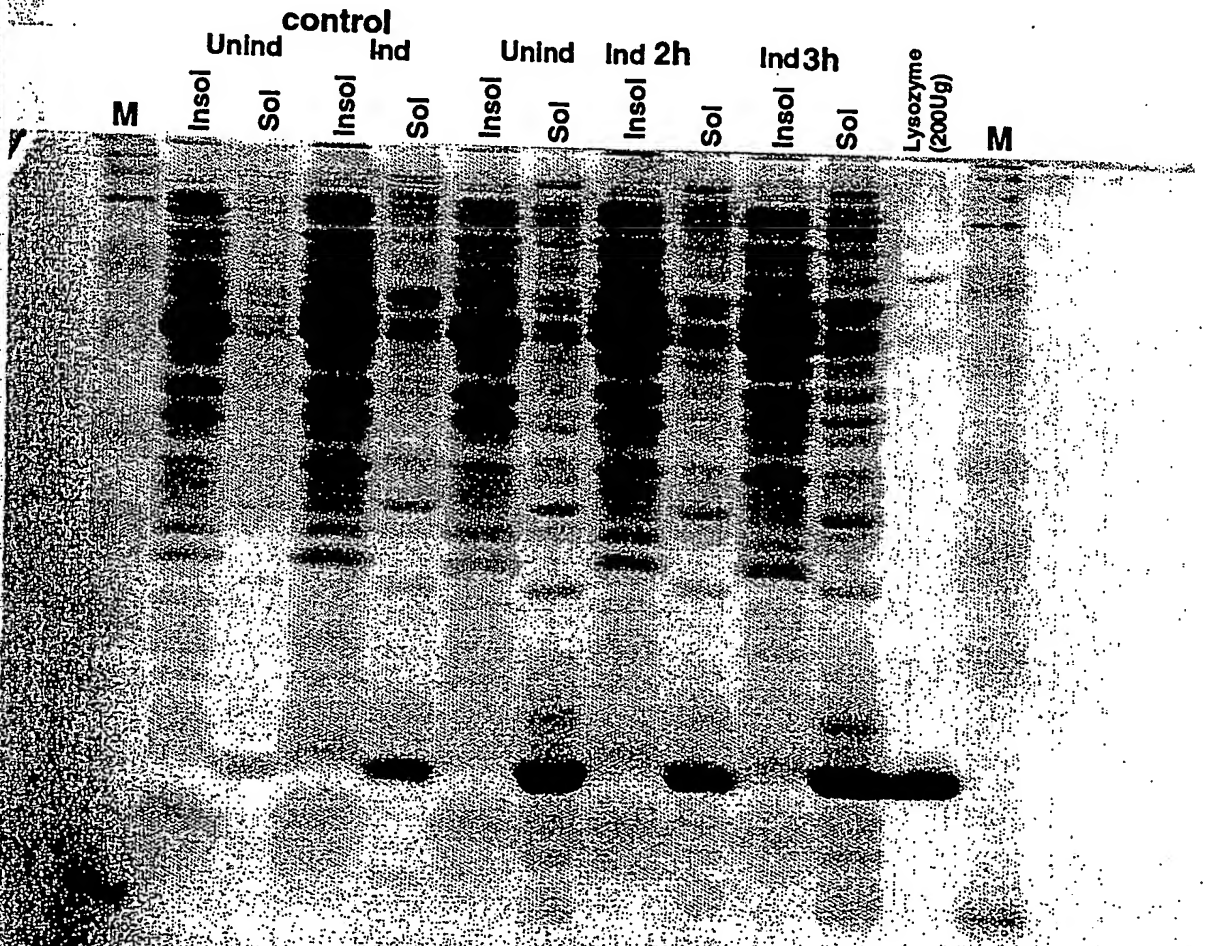


EXHIBIT 62

Name: Jagathpala Shetty Date: 12/15/1

Experiment: SDS-PAGE analysis

- → Marker (Gibco-prestained)
- S } control (empty vector) uninduced
- IN }
- S } control induced
- IN }
- S } C-58 uninduced
- IN }
- S } C-58 induced 2 hrs
- IN }
- insol. } C58
- sol. }
- insoluble induced 3 hrs
- ~~insoluble~~ lysozyme 20 µg
- Marker

EXHIBIT 63

Name: Jagathpala Sheth Date: 12/15/99

Experiment: Northern Blot analysis of C58

Northern Blot analysis

Probe: C-58-ORF. The PCR product of C58 ORF with Xho-I and NCO-I restriction enzyme digested was cleaved with above enzyme and purified on agarose to clean off the end fragments.

Labeling of DNA (Progesterone method)

4X of DNA (13.9ng/l)
29.5% H_2O → Boil - 5 min. in water bath
10X of OLBJ (from MJW)

↓

Keep at -20°C for a while.

↓

Add 5X of [α^{32}P] dCTP

Add 1.5X of Klenow polymerase

↓

Tick for a while

↓

Keep at 37°C O/N

EXHIBIT 64

Name: Jagathpala (Jeff) Date: 12/15/09

Experiment: Northern Blot analysis – protocol

15

I. Introduction *continued*

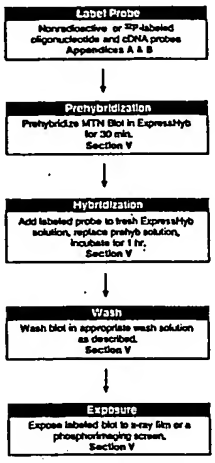


Figure 1. Overview of MTN Blot protocol. Use the β-actin probe to verify that hybridization procedures are working properly and to quantify results.

II. List of Components

Store unused MTN Blots at room temperature in a sealed plastic bag away from light.

Store used MTN Blots at 4°C in a sealed plastic bag until needed.

Store control probe at –20°C.

- 1 MTN Blot
- 100 ng Human β-actin cDNA control probe (2.0 kb) in 20 µl of TE buffer (pH 7.5). Sufficient for 2–4 labeling experiments.
- 25 ml ExpressHyb™ Hybridization Solution

III. Additional Materials Required

- 20X SSC
  - 3 M NaCl
  - 0.3 M Sodium citrate (pH 7.0)
- Wash Solution 1
  - 2X SSC
  - 0.05% SDS
- Wash Solution 2
  - 0.1X SSC
  - 0.1% SDS
- Wash Solution 3
  - 2X SSC
  - 0.1% SDS

V. Hybridization of Oligonucleotide & cDNA Probes

For hybridizing radioactively-labeled probes follow Section A. For hybridizing nonradioactively-labeled probes follow Section B.

A. Hybridization of radioactively-labeled probes

We recommend the following probe concentrations:

- cDNA probes: 2–10 ng/ml or 1–2 x 10^6 cpm/m.
- Oligonucleotide probes: 20–50 ng/ml or 1–2 x 10^7 cpm/ml.

Note: Higher probe concentrations will reduce hybridization time, but may increase background.

1. Warm ExpressHyb Solution at 68°C, and stir well to completely dissolve any precipitate. For oligonucleotide probes, equilibrate ExpressHyb at 37°C.
2. Prehybridize membranes in a minimum of 5 ml of ExpressHyb Solution, with continuous shaking for 30 min at the appropriate temperature:
   For cDNA probes: 68°C
   For oligonucleotide probes: 37°C

   Note: If you are using hybridization bottles, make sure that the marked side of the membrane is flush against the side of the bottle. Bubbles between the membrane and the bottle can give the appearance of bubbles on the blot.
3. Denature radioactively labeled probes at 95–100°C for 2–5 min. Then chill quickly on ice.
4. Add radiolabeled probe to 5 ml of fresh ExpressHyb, and mix thoroughly.
5. Replace the ExpressHyb Solution with the fresh solution containing the radiolabeled probe. Remove all air bubbles from the container, and make sure ExpressHyb Solution is evenly distributed over the blot.
6. Incubate with continuous shaking for 1 hr at the appropriate temperature:
   For cDNA probes: 68°C
   For oligonucleotide probes: 37°C
7. Rinse the blot in Wash Solution 1 several times at room temperature. Wash for 30–40 min with continuous agitation; replace the wash solution several times.
8. Wash the blot two times in Wash Solution 2 with continuous shaking for 40 min at the appropriate temperature:
   For cDNA probes: 50°C
   For oligonucleotide probes: room temperature
9. Remove the blot with forceps and shake off excess wash solution.

   Note: Do not allow the membrane to even partially dry. Allowing the membrane to dry can cause high background and will make subsequent probe removal difficult.

V. Hybridization Protocols *continued*

10. Immediately cover the blot with plastic wrap. Mount on Whatman 3 MM Chromatography paper. Wrap again with plastic wrap.
11. Expose the MTN Blot using a phosphorimaging screen. The Storm® PhosphorImager (Molecular Dynamics) is suitable for this application. Alternatively, expose to x-ray film at –70°C with two intensifying screens.
12. Strip probe from the blot by incubating the blot in sterile H_2O containing 0.5% SDS as outlined below.
    a. Heat the sterile H_2O/0.5% SDS solution to 90–100°C.
    b. Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
    c. Incubate for 10 min, shaking frequently.
    d. Allow the H_2O to cool for 10 min before removing the blot.
    e. Remove the blot and air-dry until it is dry enough to be slipped into a plastic bag. The membrane can be stored at –20°C until needed.

EXHIBIT 65

Name: Jagathpal Shetti Date: 10/16/99
Experiment: Northern Blot analysis

Probe were purified in 0.5 ml of eluting buffer, denatured at 95°C & chill on ice.

~~Added to 7 ml of the~~

After time the membrane (from

(1) A Mandal, - MTN - Blot from Clontech - used once, stripped dried) was incubated with 7 ml of pre hyb soln at 68°C in a plastic bag (sealed) for 1 1/2 hrs. (carefully shake [illegible])

(2) Purified probe added to 7 ml of exp. hyb. ~~and~~. The plastic bag was emptied and the ~~solution was~~ bag filled with the solution. Sealed carefully and incubated at 68°C. 1 1/2 hrs.

(3) Discard the exp hyb soln ~~and~~ ~~wash~~ place it on a dish and wash several times with wash bf 1. (2x SSC, 0.05% SDS) and incubate with the same for 40 min. Replace the wash soln 3 times (temp. RT)

(4) Replace with wash soln 2. (0.1x SSC, 0.1% SDS, 50°C) 40 min - 3 changes.

(5) Take the Blot in little amount of

Name: Jagathpal . Shetti Date: 12/16, 19

Experiment: Western Blot analysis of C58

wash buffer. Place it on a platform made out of a whatman paper and a saran wrap.

Place another saran wrap on the top of the ~~gel~~ blot immediately (so as not allow it to be dried). Place the marker spots on the edges.

Place the blot inside the cassette. with intensifying screen. expose for 18 hrs.

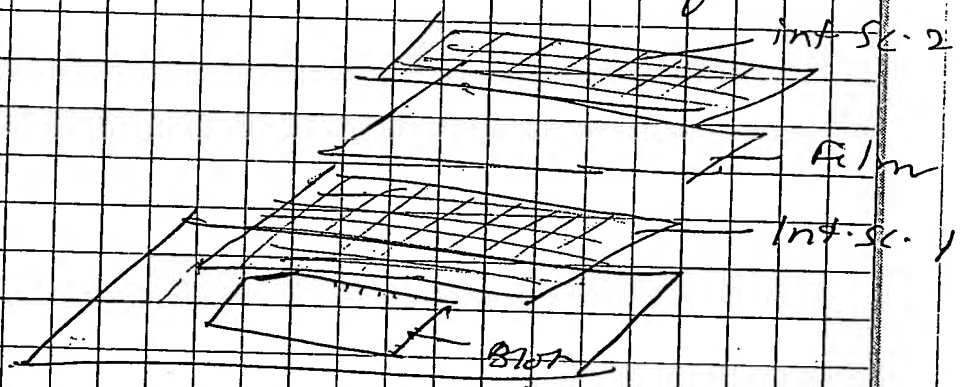
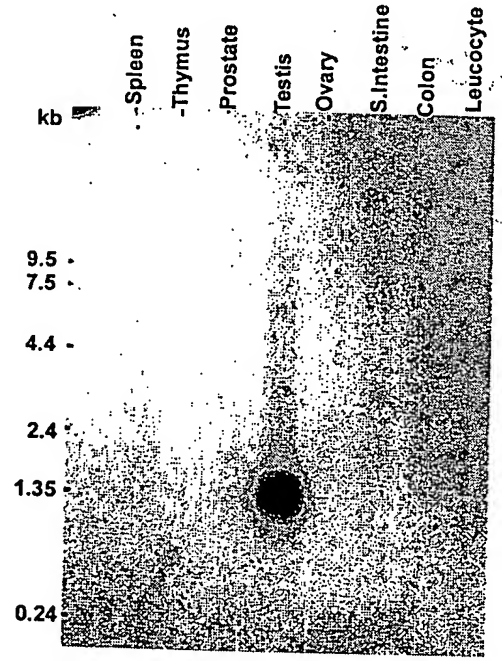


EXHIBIT 67

MULTIPLE TISSUE NORTHERN
CLONTECH
developed on 12-17-99.



C58 is expressed only in testis!